Familial Melanoma - mutation of p16 (INK4)

\[ \text{p16} \rightarrow \text{CDK4} \rightarrow \text{Cyclin D} \]

\[ \text{inactive} \rightarrow \text{Cyclin D} \rightarrow \text{CDK4} \rightarrow \text{Rb} \rightarrow \text{p} \rightarrow \text{E2F (active)} \]

p16 promoter has p53 binding site (much for arrest of G1 → S)

p16 also mutant in 50-70% of certain Tis'

Familial Non-polyposis colon ca. (NPCC)

Most: mutation in MSH2 gene

\[ \text{detection mismatched DNA bases (part of DNA repair)} \]

Find MSH2 mutations in sporadic colon ca. too

Other NPCC families: mutations in other genes in mismatch DNA repair
Familial Breast Ca - mutations of BrCa1, BrCa2

BrCa1

- High breast, ovarian Ca (\( \geq 50\% \) likelihood of BC in unaffected women)
- High T in Ashkenazi Jewish women
- \( \Rightarrow \) Screening, prophylactic mastectomy
- BrCa1 + 2 proteins involved in DNA repair

Detecting Tu Su genes in sporadic tumors

i.e.

\[
\text{TuSu}^+ / \text{TuSu}^+ \quad \rightarrow \quad \neg \neg
\]

Predominant mech for TuSu loss

\[
\begin{align*}
\text{TuSu} & \quad \rightarrow & & \text{deletion} & \quad \rightarrow \\
\text{Normal Cell} & & & \text{(small deletion)} & \quad \text{Tumor}
\end{align*}
\]

Detection & look for "loss of heterozygosity" (LOH) in Tu DNA
Detecting LOH: Need molecular markers that detect a polymorphism between maternal and paternal alleles.

\[
a \times b \rightarrow \text{Normal Cell} \quad \text{Tumor}
\]

RFLP (Restriction Fragment Length Polymorphism) probes probe: cloned cellular DNA that is also a common polymorphism that can be distinguished by restriction enzyme sites for a particular enzyme.

\[
a \downarrow \quad \text{GATTC} \quad \downarrow
\]

↓ Southern blot, using RFLP probe + restriction enzyme.

\[
\begin{align*}
a/a & \quad a/b & \quad b/b \\
\text{-} & \quad \text{-} & \quad \#\#
\end{align*}
\]

RFLP probes developed for all human chromosomes (deletions, regions), locations already known.
for analysis, for each patient use RFLP probes that are informative for that patient

Use of RFLP probes to identify LOH in a tumor

\[ \begin{array}{ccc}
2a^+ & b^+ & 2a^+ + b^- \\
1a^- & b^- & 1a^- \\
T.S. & X & T \\
\end{array} \]

from location of RFLP probes on chromosome, map LOH site

Common LOH in particular kind of Ca \Rightarrow loss of a Tu Su

Several new Tu Su's found from common LOHs

MCC - mutated in colon Ca

DCC - deleted in colon Ca

PTEN - PIP3 phosphatase

P.Tesz 4,5 BP \xrightarrow{P13K} PIP3 \rightarrow pAKT \xrightarrow{?} \text{PTEN}
More convenient markers to detect LOH + polymorphisms

Simple sequence length polymorphisms (SSLP's)

Genomic DNA

\[ 5'-10 \text{bp} \]

# of repeats frequently differ for different people

SSLP probes: PCR primers surrounding SS repeat

(There's a database of such primer pairs mapped onto human, mouse genomes)

[Diagram]

Can use informative SSLP probes to look for LOH

\[ \frac{N}{T} \]

SNPs - single nucleotide polymorphisms mapped in human genome

a

b

c

[PCR three regions, cut + DNA, seq. at N, T, DNA]
Multiple changes in Tumors

Normal → proto-onc. activation → Tumor Cell

USp inactivations

e.g. colon Ca

Kras activation

p53 mutation

APC or Mcc inactivation

Dcc inactivation

As polype progress to Ca